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Effect of Decoction the Bark of the Root of *Sclerocarya Birrea* (A. Rich) Hochst (Anacardiaceae) on Memory in White Mice *Mus Musculus* Swiss (Murideae)

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Abstract: The present study evaluates the pharmacological effects of *Sclerocarya birrea* (*S. birrea*) decoction on memory in white mice. T-maze tests with and without induction of memory loss were performed one hour after oral administration of different doses of the decoction (410 ; 205 and 102.5 mg/kg) to assess the behavioral performance of the animals in the T-maze. In the T-maze test without induction of memory loss, administration of the decoction resulted in a significant decrease in latency of choice of the preferred arm and time spent in the discriminated arm. In the T-maze test with induction of memory loss by scopolamine, S. birrea decoction reversed the effects of scopolamine in mice, significantly enhancing cognitive performance. Doses of 102.5 and 205 mg/kg of the decoction led to a significant reduction in the latency of choice of the preferred arm and the number of returns to the starting arm. The results obtained suggest that S. birrea decoction possesses properties that facilitate memorization and correct memory deficits in mice, thus justifying its use in traditional medicine for the treatment of cognitive disorders. Preliminary phytochemical characterization tests revealed the presence of alkaloids, tannins, flavonoids, triterpenes or steroids, saponins, anthraquinones and polyphenols.
Keywords: *Sclerocarya birrea*, decoction, neuroprotection, memory, white mouse.

INTRODUCTION

Memory is one of the cognitive functions of the brain that enables humans to interact with the world[1, 2]. It is the ability to record, encode, store and retrieve information by situating it in time. The set of experiences stored in the brain determines our identity[3]. However, memory can become vulnerable to internal or external cerebral stresses. Memory loss is a major cognitive disorder characterized by behavioral disorders, loss of interest and motivation[4, 5]. To this day, it remains a major public health problem. Indeed, this pathology affects more than 46 million people worldwide, all sexes and ages combined, with an estimated annual medical cost of 283,373 billion CFA francs, and an incidence of 7% of deaths per year[6, 7]. Studies have shown that the use of medicinal plants can effectively improve learning ability and memory function. These plants can boost memory and prevent cognitive pathologies associated with normal aging[8, 9]. Although many diagnostic and treatment strategies based on effective conventional drugs have been established, these are less accessible to all populations due to socio-economic conditions and present major side effects on memory[10]. For example, patients with memory loss present enormous difficulties in memory tasks requiring effort, such as learning a memory and recalling it[11, 12].

It would therefore be important to prevent the onset of memory loss by developing new categories of herbal medicines that not only have fewer side effects, but can also be beneficial for associated pathologies[13]. According to the WHO, the use of medicinal plants is widespread and of growing health and economic importance[14]. Pharmacological studies of medicinal plants could be one of the most promising alternatives, and it is with this in mind that the present study was carried out. The aim of this study was to evaluate the pharmacological effects of *S. birrea* decoction on memory capacity in white mice. The aim was to provide scientific proof of the therapeutic efficacy attributed to this plant from the traditional pharmacopoeia.

MATERIALS AND METHODS

Plant material and decoction preparation

Samples of *S. birrea*, harvested in May 2023 in southern Chad, were identified at the Yaoundé National

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Herbarium in Cameroon under reference 2748SFR. After harvesting, *S. birrea* root bark was washed with water and dried at room temperature in the shade, then crushed using a mortar and finally sieved using a 0.5-mm mesh sieve to obtain the powder needed to prepare the decoction. The powder obtained is stored at room temperature in a closed jar.

Next, 2.5 grams of the *S. birrea* powder obtained earlier was transferred to a beaker containing 50 mL of distilled water. The mixture was boiled for 30 minutes on a hot plate set at 100°C. After cooling, the mixture was filtered through a Wattman n°1 filter paper, and the filtrate recovered constituted the stock solution.

In order to determine the mass yield of the decoction, the filtrate obtained (stock solution), whose volume was 23 mL, was evaporated in an oven at 40°C. A mass of 0.9435 g of dry *S. birrea* decoction was obtained in 37.74 % yield, with a stock solution concentration of 41 mg/mL. Given an administration volume of 10 mL/kg, the initial dose of the decoction is 410 mg/kg. Two other solutions with different doses (102.5 and 205 mg/kg) were prepared from the dry decoction.

Phytochemical characterization tests

Preliminary phytochemical characterization tests were carried out using qualitative colorimetric methods[15], to determine the main chemical groups.

Alkaloid test: 1 mL of the decoction, prepared at 0.3 mg/mL, was taken into a test tube, then 1 mL of HCl, prepared at 5%, and 3 drops of Dragendorff's reagent were added. The formation of a white or orange precipitate indicates a positive test[16].

Tannin test: 1 mL of the decoction, prepared at 0.3 mg/mL, was taken into a test tube and 3 drops of FeCl3, prepared at 1%, were added. The formation of a blue-blackish coloration confirmed the presence of tannins[17].

Flavonoid test: 1 mL of the decoction, prepared at 0.3 mg/mL, was taken into a test tube, then 3 magnesium chips and 1 mL concentrated hydrochloric acid were added. The formation of an orange coloration indicates the presence of flavones, red that of flavonols and violet that of flavonones[18].

Test for triterpenes or steroids: 1 mL of the decoction, prepared at 0.3 mg/mL, was taken in a test tube. 1 mL chloroform, 1 mL concentrated H2SO4 and 1 mL acetic anhydride were added. The formation of a violet or blackish-green coloration confirms that the test is positive[18].

Anthraquinone assay: 1 mL of the decoction, prepared at 0.3 mg/mL, was taken into a test tube. 2 mL mixture (petroleum ether/chloroform) and 2 mL NaOH, prepared at 10%, were added to the decoction. The formation of a red coloration indicates a positive test[18].

Phenolics test: 1 mL of *S. birrea* decoction prepared at 0.3 mg/mL was taken into a test tube and 3 drops of FeCl3 prepared at 10% were added. The formation of a green or bluish coloration confirms the presence of phenolic compounds[19].

Behavioural tests

Experimental animals

Naïve white mice, *Mus musculus* Swiss (Murideae), of both genera and weighing between 18 and 25 g were used in our experiments. The mice were supplied by the National Veterinary Laboratory (NAVETLA) in Garoua. They were acclimatised for one week at the Medicinal Plants, Health and Galenic Formulation Laboratory of the Biological Sciences Doctoral Training Unit of the University of Ngaoundéré before the start of the experiments. These animals were housed in standard cages and consumed tap water and granules ad libitum. The experiments were conducted in accordance with the guidelines of the Cameroon National Ethics Committee (N° FWA-IRB00001954, October 22 1987).

Effect of *Sclerocarya birrea* decoction on memory in T-maze mice

The effects of *S. birrea* on the level of exploration, learning and memory in naïve mice (unmanipulated mice) placed in the T-maze were evaluated. Two days before the start of the experiments, the animals were progressively deprived of food to maintain them at 80-85% of their body weight. The animals were divided into 6 homogeneous batches of 5 animals each. The animals received distilled water for the negative control batch, Diclofenac (5 mg/kg) for the positive control batch and different doses of *S. birrea* decoction for the test batches. Mice were placed one after the other in the starting arm of the T-maze one hour after administration of the different substances. This task was performed in three phases: habituation, acquisition and retention[20].

In the first or housing phase, the mice are familiarized with the device for a period of 5 minutes. Food is placed in each corridor to encourage exploration. The experimenter places the mice in the starting compartment. After 15 seconds, he opens all the guillotine doors. The animal can then choose one or other of the lanes, indicating its preference. The parameters recorded were: latency time to choose an arm (the arm that will be the animal's preferred arm throughout the test), time spent and number of entries into the preferred arm and the discriminated arm, and number of returns to the starting arm.

The second or acquisition phase begins 24 hours after the habituation phase. The corridor of the arm discriminated by the animal is closed, then a reinforcer

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(food) is placed in the arm chosen by the animal. The experimenter places the mouse in the starting compartment and lets it move towards the open corridor (preferred corridor during habituation). This phase takes 5 min for each animal. The following parameters are recorded: latency to retrieve food, time spent and number of entries into the preferred arms, and number of returns to the starting arm.

Finally comes the retention phase, 24 hours after the acquisition phase. Each animal is placed in the device for 5 min, this time with all arms open. The experimenter places food in both arms of the maze. The parameters recorded are: latency time to find the preferred arm, time spent and number of entries in the preferred arm and the discriminated arm, and number of returns to the starting arm. After 5 min of experimentation

Effect of *Sclerocarya birrea* decoction on memory in scopolamine-treated mice in the T maze

The effects of *S. birrea* on scopolamine-induced memory loss were assessed in naïve mice placed in the T-maze, with animals divided into 6 homogeneous batches of 5 mice. They were divided as follows : 1 negative control batch receiving only distilled water (10 mg/kg), 1 scopolamine control batch receiving distilled water (10 mg/kg) and scopolamine (0.1 mg/kg) one hour later, 1 positive control batch receiving diclofenac (5 mg/kg) and scopolamine (0.1 mg/kg) one hour later, the test batches received the different doses of the *S. birrea* decoction (102.5, 205 and 410 mg/kg) and scopolamine (0.1 mg/kg) one hour later. However, these administrations took place only during the retention phase. During the dwelling and acquisition phase, the animals were subjected to training. Mice were placed one after the other in the starting arm of the T-maze, 30 minutes after scopolamine administration for positive control animals and test batches, and 30 minutes after distilled water administration for negative control animals. The same parameters are recorded as in the normal T-maze for the dwelling, acquisition and retention phases.

Analysis of Results

Data were expressed as means ± standard deviations. Analysis of variance (ANOVA) was used for comparison of means, followed by Dunnett and Turkey's test for comparison of variables using XL. Stat version 2007 software. Differences were considered significant at $p \leq 0.05$.

RESULTS

Phytochemical screening of *Sclerocarya birrea* decoction

Qualitative characterization of *S. birrea* decoction reveals the presence of the following major chemical families: phenolic compounds, tannins, anthraquinones, alkaloids, saponins and triterpenes, which are listed in Table 1 below.

Table 1 : Main chemical families of *Sclerocarya birrea* decoction

Chemical compound families	Existence
Alkaloids	++
Flavonoids	+
Saponins	+
Tannins	+++
Triterpenes	+++
Anthraquinones	++
Steroids	++
Polyphenols	+++

Legend: + = Present; ++ = Abundant; +++ = Very abundant

Memory effects of *Sclerocarya birrea* decoction on mice in the T-maze test

Habituation phase

Effects on latency to enter the arrival arm

A significant reduction ($P < 0.001$) in latency was observed in mice treated with different doses of *S. birrea* decoction, compared with those in the normal control group (Figure 1). The latency time fell from 9.6 ± 0.72 seconds in mice from the normal control batch to 1.8 ± 0.96 seconds in those treated with the 410 mg/kg dose of the decoction. Similarly, diclofenac significantly ($P < 0.001$) reduces this time to 2.8 ± 0.64 seconds compared with the normal control batch.

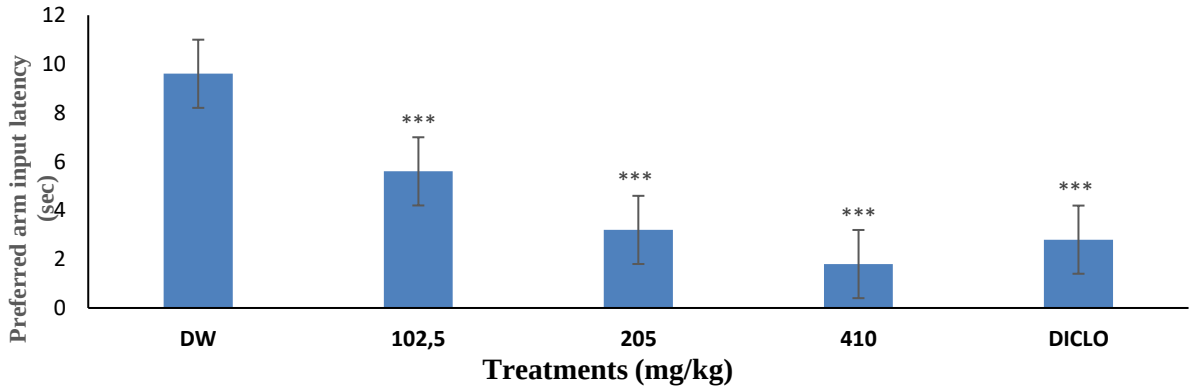

Figure 1: Effects of *Sclerocarya birrea* decoction on latency to select the preferred arm in the T maze.

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Each column represents mean ± SEM of five animals. The data analysis was performed using one way ANOVA followed by Turkey multiple comparisons test. ***p < 0,001. DW, Distilled water; 102.5; 205 and 410: *Sclerocarya birrea* doses in mg/kg ; DICLO, Diclofenac.

Effects on the Number of Returns to the Starting Arm

A significant reduction (P < 0.001) in the number of returns to the starting arm in mice treated with the various doses of *S. birrea* decoction compared with those in the normal control batch is also noted in Figure 2 opposite. This number varies from 6.2 ± 0.64 in the normal control batch to 1.8 ± 0.64 corresponding to the 410 mg/kg dose of *S. birrea* decoction. Similarly, diclofenac significantly (P < 0.001) reduces this number to 2.8 ± 0.72 compared with the normal control batch.

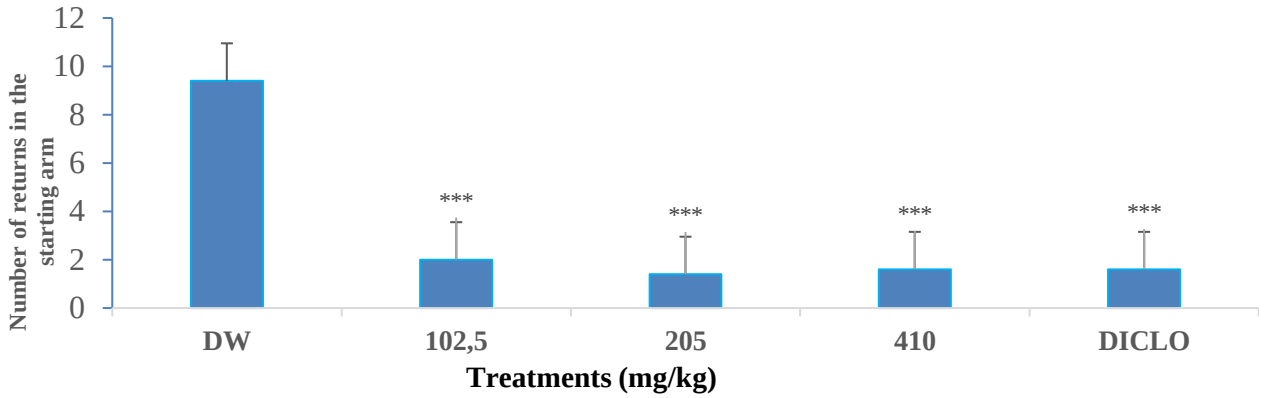

Figure 2: Effects of *Sclerocarya birrea* decoction on the number of returns to the starting arm in the T maze

Each column represents mean ± SEM of five animals. The data analysis was performed using one way ANOVA followed by Turkey multiple comparisons test. ***p < 0,001. DW, Distilled water; 102.5; 205 and 410: *Sclerocarya birrea* doses in mg/kg; DICLO, Diclofenac.

Effects on time spent in the preferred arm of the T maze

A significant increase (P < 0.001) in the time spent in the preferred arm was observed in animals receiving the different doses of *S. birrea* decoction compared with those in the normal control batch (Figure 3). This time increased from 48.4 ± 0.88 seconds in normal control mice to 111.2 ± 0.96 seconds corresponding to the 410 mg/kg dose of *S. birrea* decoction. Diclofenac increases this time significantly (P < 0.001) to 68.2 ± 0.64 seconds compared with the normal control batch.

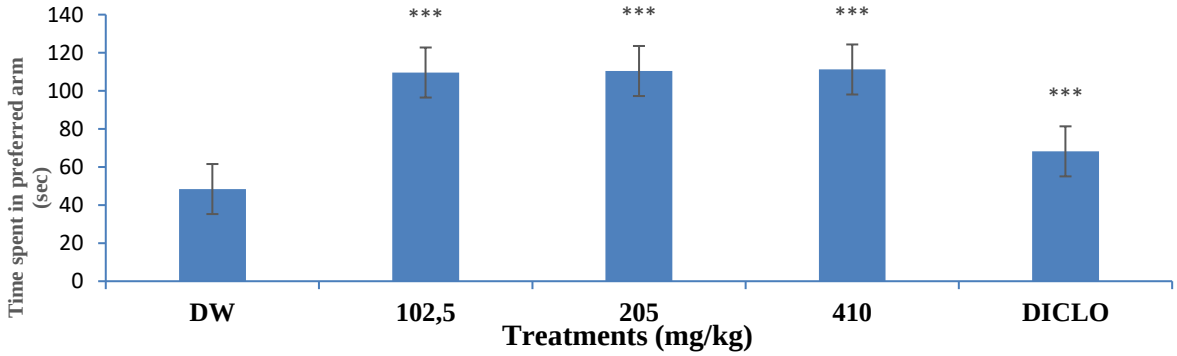

Figure 3: Effects of *Sclerocarya birrea* decoction on time spent in the preferred arm of the T maze

Each column represents mean ± SEM of five animals. The data analysis was performed using one way ANOVA followed by Turkey multiple comparisons test. ***p < 0,001. DW, Distilled water; 102.5; 205 and 410: *Sclerocarya birrea* doses in mg/kg; DICLO, Diclofenac.

Retention phase

Effect on latency to enter the first arrival arm

Figure 4 shows a significant decrease (P < 0.001) in the latency to enter the first arrival arm in mice treated with the different doses of *S. birrea* decoction, compared with those in the normal control batch. This latency time varied from 5.8 ± 0.64 seconds in mice from the normal control batch to 2.4 ± 0.48 seconds in those treated with the 205 mg/kg dose of the decoction. Similarly, diclofenac significantly (P < 0.001) reduced this time to 1.6 ± 0.72 seconds compared with the normal control batch.

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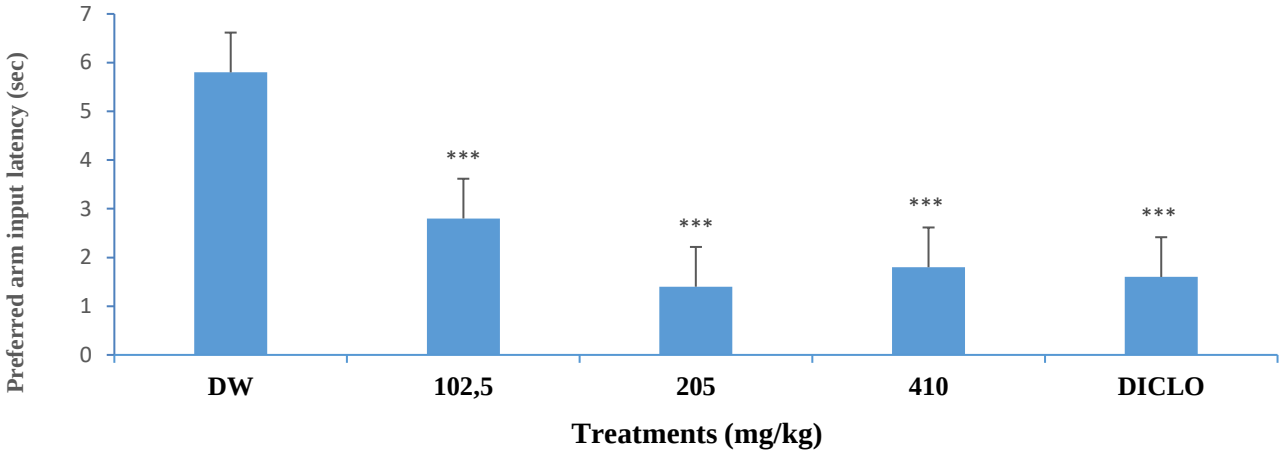

Figure 4: Effects of *Sclerocarya birrea* decoction on the number of entries in the preferred arm of the T maze

Each column represents mean ± SEM of five animals. The data analysis was performed using one way ANOVA followed by Turkey multiple comparisons test. ***p < 0,001. DW, Distilled water; 102.5; 205 and 410: *Sclerocarya birrea* doses in mg/kg; DICLO, Diclofenac.

Effects on the number of returns to the starting arm

Figure 6 shows a significant reduction (P < 0.001) in the number of returns to the starting arm in mice treated with the different doses of S. birrea extract, compared with those in the normal control batch. This number varied from 3.4 ± 0.72 in mice from the normal control batch to 1.4 ± 0.48 in those treated with the 410 mg/kg dose of the extract. Similarly, diclofenac significantly (P < 0.001) reduces this number to 1.6 ± 0.72 compared with the normal control batch.

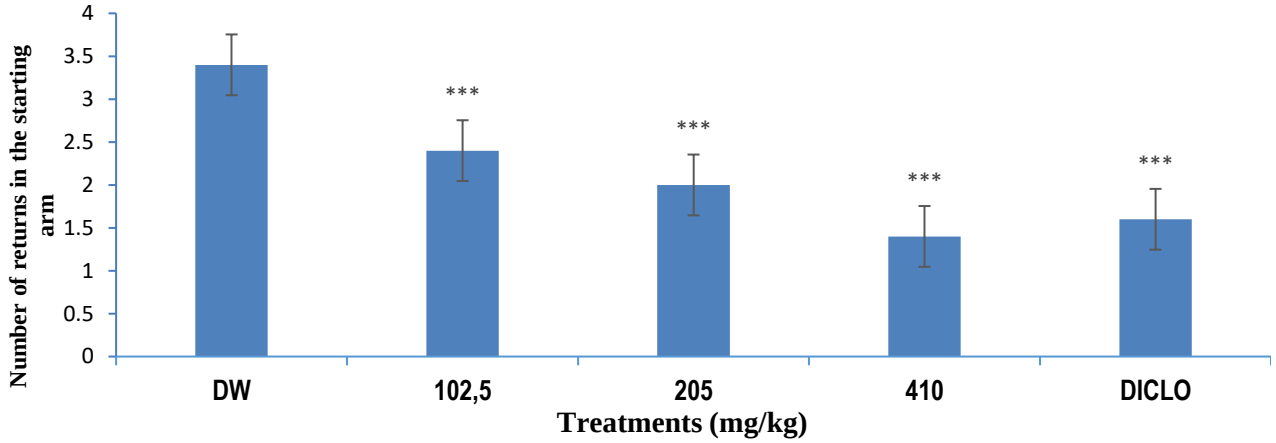

Figure 5: Effects of *Sclerocarya birrea* decoction on the number of returns in the start arm of the T maze

Each column represents mean ± SEM of five animals. The data analysis was performed using one way ANOVA followed by Turkey multiple comparisons test. ***p < 0,001. DW, Distilled water; 102.5; 205 and 410: *Sclerocarya birrea* doses in mg/kg; DICLO, Diclofenac.

Effects on time spent in the arrival arms (preferred and discriminated) in the T maze

Figure 5 shows a significant increase (P < 0.001) in the time spent in the preferred arm in mice treated with *S. birrea* decoction compared with those in the normal control group. Time spent in the preferred arm increased from 106 ± 0.40 seconds in normal control mice to 203.8 ± 0.64 seconds in those treated with 102.5 mg/kg of the decoction. Similarly, diclofenac significantly (P < 0.001) increased this time to 121.6 ± 0.88 seconds compared with the normal control batch. On the other hand, a significant decrease (P < 0.001) in the time spent in the discriminated arm was observed in mice treated with *S. birrea* decoction compared to those in the normal control batch. The time spent in the discriminated arm varied from 101.6 ± 0.48 seconds in mice from the normal control group to 20 ± 0.40 seconds in those treated with 205 mg/kg of the decoction. Similarly, diclofenac significantly (P < 0.001) reduced this time to 28.4 ± 0.48 seconds compared with the normal control batch.

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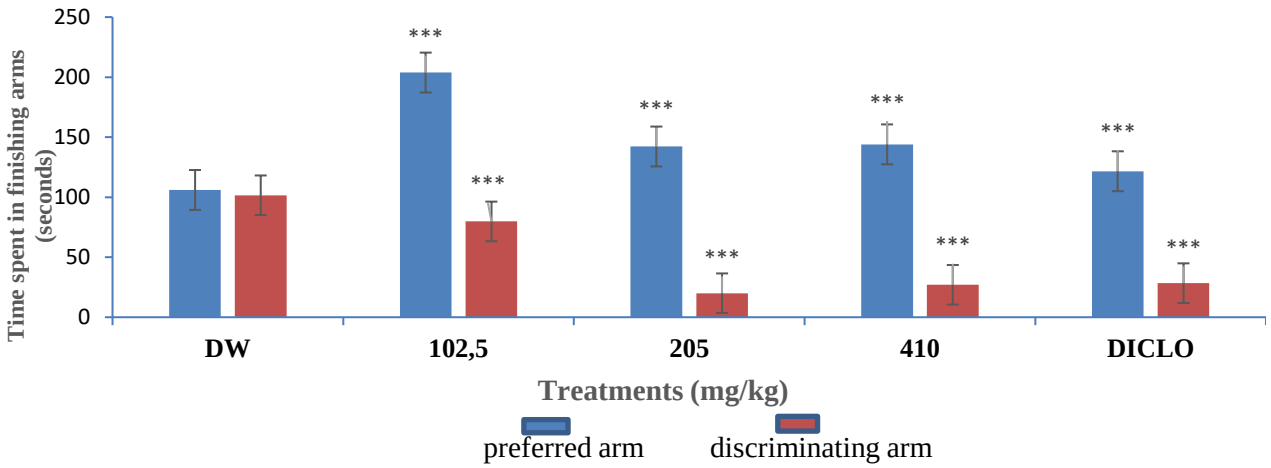

Figure 5: Effects of *Sclerocarya birrea* decoction on time spent in the arrival arms (preferred and discriminated) in the T maze

Each column represents mean ± SEM of five animals. The data analysis was performed using one way ANOVA followed by Turkey multiple comparisons test. ***p < 0,001. DW, Distilled water ; 102.5; 205 and 410 : *Sclerocarya birrea* doses in mg/kg ; DICLO, Diclofenac.

Protective effects of *Sclerocarya birrea* decoction on memory with scopolamine induction in mice in the T-maze test

Retention phase

Effects on latency to enter the first arrival arm of the T-maze

Figure 6 shows a significant increase (P < 0.001) in the latency to enter the first arrival arm, from 7.2 ± 0.64 seconds in the normal control group to 10.8 ± 0.64 seconds in the negative control group. *S. birrea* decoction antagonized the effects of scopolamine, significantly (P < 0.001) reducing this time to a minimum value of 2.6 ± 0.48 seconds in animals receiving 205 mg/kg. Diclofenac significantly (P < 0.001) reduces this time to 3 ± 0.4 seconds compared with the negative control batch.

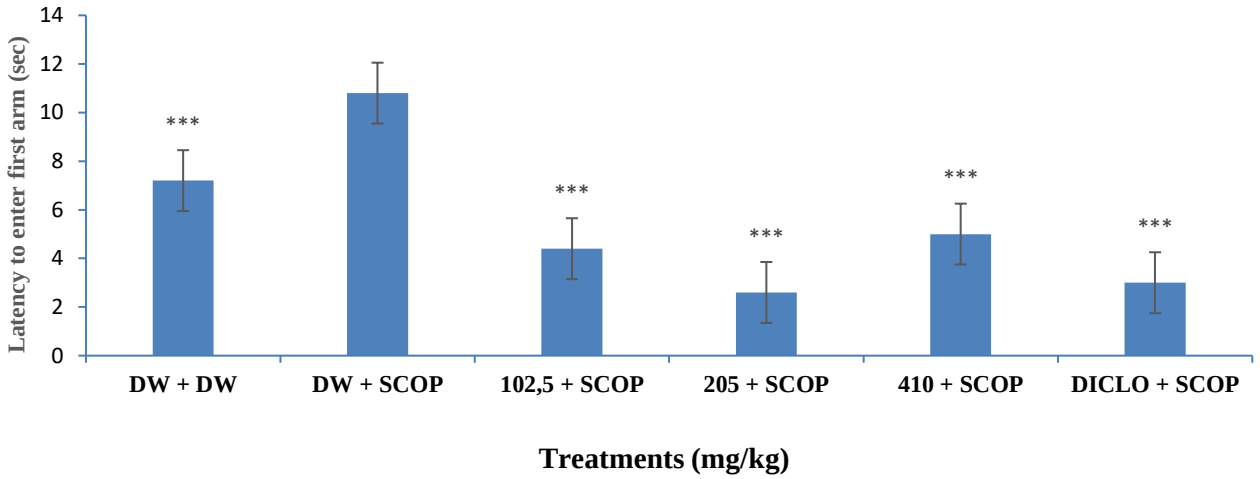

Figure 6: Effects of *Sclerocarya birrea* decoction on scopolamine-induced latency in the T-labyrinth

Each column represents mean ± SEM of five animals. The data analysis was performed using one way ANOVA followed by Turkey multiple comparisons test. ***p < 0,001. Scopolamine group (DW + SCOP). DW, Distilled water; 102.5; 205 and 410: *Sclerocarya birrea* doses in mg/kg; SCOP, Scopolamine; DICLO, Diclofenac.

Effects on the number of returns to the starting arm of the T maze

Figure 7 shows that scopolamine significantly (P < 0.001) increases the number of returns to the starting arm, which varies from 4.6 ± 0.48 in normal control mice to 10.4 ± 0.48 in negative control mice. *S. birrea* decoction inhibits the effects of scopolamine, significantly (P < 0.001) reducing this number to a minimum value of 1.8 ± 0.32 in animals treated with 410 mg/kg. However, diclofenac significantly (P < 0.001) reduced this number to 2.4 ± 0.48 compared with the negative control batch.

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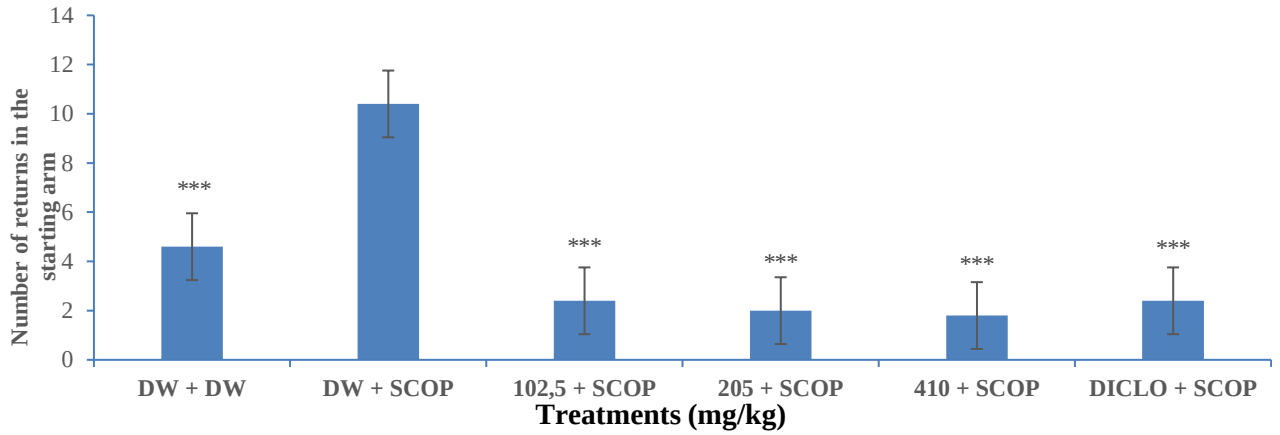

Figure 7: Effects of *Sclerocarya birrea* decoction on the number of returns to the starting arm of the T maze

Each column represents mean ± SEM of five animals. The data analysis was performed using one way ANOVA followed by Turkey multiple comparisons test. ***p < 0,001. Scopolamine group (DW + SCOP). DW, Distilled water ; 102.5; 205 and 410 : *Sclerocarya birrea* doses in mg/kg ; SCOP, Scopolamine ; DICLO, Diclofenac.

Effects on time spent in the arrival arms (preferred and discriminated) of the T-maze

Figure 8 shows that scopolamine administration induces a significant decrease (P < 0.001) in the time spent in the preferred arm, from 62.4 ± 0.88 sec in normal control mice to 49.6 ± 0.88 sec in negative control mice. *S. birrea* decoction counteracts the effects of scopolamine by significantly (P < 0.001) increasing this time to an optimum value of 119 ± 0.4 sec in animals treated with 205 mg/kg. Similarly, diclofenac significantly (P < 0.001) increased this time to 113.8 ± 0.64 seconds compared with the negative control batch. On the other hand, the time spent in the discriminated arm increased significantly (P < 0.001) from 37.2 ± 0.96 seconds in the normal control group to 72 ± 0.40 seconds in the negative control group. *S. birrea* decoction inhibited the effects of scopolamine, significantly (P < 0.001) reducing this time to an optimum value of 29.4 ± 0.88 seconds in animals receiving 205 mg/kg of the plant decoction. Similarly, diclofenac significantly (P < 0.001) decreased this time to 28.8 ± 0.64 seconds compared with the negative control batch.

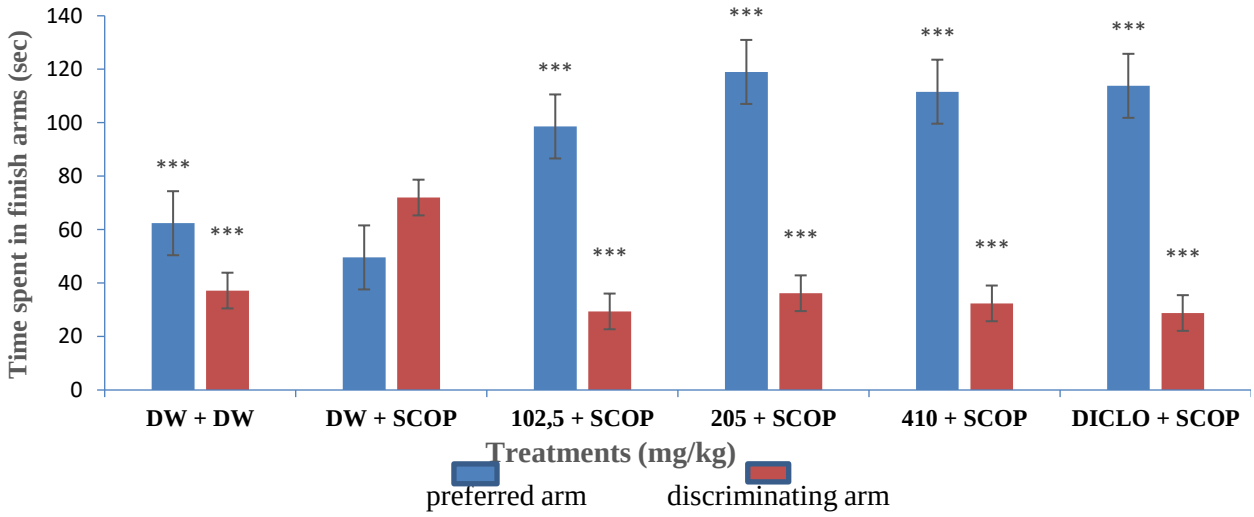

Figure 8: Effects of *Sclerocarya birrea* decoction on time spent in the arrival arms (preferred and discriminated) of the T maze

Each column represents mean ± SEM of five animals. The data analysis was performed using one way ANOVA followed by Turkey multiple comparisons test. ***p < 0,001. Scopolamine group (DW + SCOP). DW, Distilled water; 102.5; 205 and 410: *Sclerocarya birrea* doses in mg/kg; SCOP, Scopolamine; DICLO, Diclofenac.

DISCUSSION

Memory is a set of neurological processes for acquiring, storing and recalling past experiences and information[21, 22]. It is one of the cognitive functions of the brain that enables humans to interact with the world[23, 24]. However, memory can become vulnerable in the case of

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cognitive disorders or neurodegenerative diseases. Studies have shown that the use of certain medicinal plants can effectively improve learning ability and memory function. These plants can boost memory and prevent age-related cognitive pathologies[25]. Indeed, memory drugs are used to improve memory capacity in patients suffering from memory loss and other reasoning-related illnesses[26].

However, the present study demonstrated a decrease in latency, time spent in the discriminated arm and number of returns to the starting arm, on the one hand, and an increase in the number of entries and time spent in the preferred arm, as well as the number of entries in the discriminated arm, on the other hand, in mice given *S. birrea* decoction. The decrease in food retrieval latency suggests an improvement in reference memory. Similarly, the increase in the number of entries into the arrival arms indicates an increase in exploration. In addition, improved memory capacity and reduced stress due to the presence of food reinforcers are conducive to memory function. Similar results were obtained by Heru and his collaborators in 2017[27]. It can therefore be said that *S. birrea* decoction has properties that facilitate learning and memory during the different phases of the test.

To confirm the positive effects of *S. birrea* decoction on memory, the scopolamine memory loss induction test was used. The results showed a reduction in the number of returns to the start arm, the time spent in the arrival arms and the latency to retrieve food in mice given different doses of the plant decoction, compared with those in the negative control group. *S. birrea* decoction was shown to significantly reverse the effect of scopolamine by increasing learning and memory capacity in the animals. Similarly, the increase in the number of entries and the time spent in the arrival arms implies an increase in exploration, and hence in mnesic faculties and reference memory, in mice given different doses of *S. birrea*[28, 29].

Similarly, *S. birrea* extract was found to contain phenolic compounds such as polyphenols, tannins and triterpenes, which respond effectively to antioxidant activity. These results corroborate those of Dai and Mumper, (2010) in their studies on the extraction of plant phenols and the analysis of their antioxidant and anti-cancer properties.

CONCLUSION

At the end of this study, we can conclude that *S. birrea* decoction reverses the effect of scopolamine antagonist compounds by increasing the animals' memory capacity. These observations enable us to understand, at least in part, the use of *S. birrea* root bark in traditional medicine. Although further investigations are required, these results show that *S. birrea* decoction can be used in the treatment of memory impairment in traditional medicine.

Conflict of interest: No conflict of interest exists between the authors

Author contribution: All authors have contributed equally their best to produce the article in this form.

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